REMARKS

This response is filed in reply to the Office Action dated May 31, 2007 ("the Office Action"). Claims 1 to 9, 11 to 15, and 21 to 53 are pending. Of these, claims 11 to 15 and 21 to 46 have been withdrawn from consideration for covering a non-elected invention. Thus, claims 1 to 10 have been examined on the merits. Claims 10 and 16 to 20 have been cancelled without prejudice. Claims 47 to 53 are newly added.

Applicants have amended claims 1 and 6 to more clearly set forth the claimed invention. The recitation of removing living cells from the surface using a non-proteolytic process and leaving the now substantially living cell-free ECM on the surface is supported in the specification, e.g., at page 19, last full paragraph, and in claim 10 (now cancelled) as previously amended. New claims 47 to 50 further define the non-proteolytic process as including treating the cells with a calcium and magnesium chelating agent, such as EGTA or EDTA and washing with a buffer lacking calcium and magnesium. These concepts are described in the application, e.g., at page 19, last full paragraph. New claims 51 and 52 recite different ways to stimulate the substantially cell-free extracellular matrix to release morphogens. These ways are recited in the application, e.g., at page 2, second paragraph. Claim 53 recites that the power of the electrical stimulation is less than 3.0 watts. This concept is supported, e.g., at page 6, line 7. Thus, these amendments add no new matter.

We will now address the Office's rejections.

35 U.S.C. § 102

The Office rejected claims 1 to 6 and 10 as allegedly anticipated by Mitchell et al., U.S. Patent No. 6,962,814 ("Mitchell"). Applicants traverse this rejection for the following reasons.

The Office acknowledges that to make this rejection the steps of independent claims 1 and 6 have not been read in a specific order. The Office then states that Mitchell grows cells to produce an ECM, applies electrical stimulation to the culture (cells and ECM) and decellularizes the ECM with proteases such as trypsin. The Office alleges that this allows "for the cells to be removed intact," citing column 19, lines 3-5, and states, "[u]pon removal of the cells from the matrix, the morphogen composition, produced by the electrical stimulation of the matrix, is also inherently collected.

Applicants respectfully disagree with several of these assertions.

First, applicants note that Mitchell uses electrical stimulation to enhance growth conditions of cells in his tissue engineered constructs, and does not provide any indication that the stimulation causes the ECM to release morphogens.

Second, the Office alleges that Mitchell removes the cells "intact." This is simply incorrect, because Mitchell's proteolytic methods of decellularization degrade cell adhesion proteins, cell-cell junction proteins, and receptor proteins on cell membranes. See, e.g., page 43, right column, first full paragraph, of Yamato et al., MaterialsToday, 42-47, May 2004 (copy attached). In addition, Mitchell himself characterizes these methods as being used to "disrupt, degrade, and/or destroy cellular components and/or modify the matrix in which the cells are embedded so as to facilitate removal of the cells and cellular components" (col. 17, lines 52-55). As a result of this proteolytic activity, the ECM becomes littered with protein fragments from both the cells and the ECM, and the cells are no longer intact. Mitchell states that his techniques "remove cellular components, while leaving the secreted proteins, e.g., collagen and elastin, substantially intact" (col. 9, lines 1-3). Thus, his techniques appear to remove the very morphogens that applicants are trying to collect. In any event, Mitchell does nothing to keep the cells alive and intact as applicants' claims require. Instead, he cares more about keeping the ECM intact and non-immunogenic, so he tries to remove any proteins and cellular components that might cause an immune reaction (see Mitchell at col. 17, lines 28-49).

Third, the Office states that a morphogen composition is "inherently collected." Nowhere does Mitchell state that he collects any fluid, much less a morphogen composition. To the contrary, it appears that Mitchell removes the ECM from the culture medium, and washes the ECM to remove any traces of the decellularization solution (col. 20, lines 1-10). Thus, rather than collecting a morphogen composition, Mitchell likely discards any medium or wash solutions. As a result, Mitchell completely lacks any teaching of the final step in applicants' claims.

Given these differences, applicants respectfully submit that Mitchell does not anticipate claims 1 or 6, or dependent claims 2 to 5. Claim 10 has been cancelled. To further distance their claims from Mitchell, applicants have amended claims 1 and 6 to specify that when the ECM is stimulated, it is "substantially living cell-free." This amendment specifies the order in which the

claimed steps must be carried out, and provides an additional clear distinction from Mitchell, who stimulates the cells and the ECM before removing any cells from the ECM. This is the opposite of the method steps of present claims 1 and 6. Thus, applicants respectfully request that the Examiner reconsider and withdraw the rejections of claims 1 to 6 under Section 102.

35 U.S.C. § 103

The Office has rejected claims 1 to 5 and 10 as allegedly unpatentable over Rieck et al., Experimental Cell Research, 1995, volume 220, pages 36-46 ("Rieck") in view of Livesey et al., U.S. Patent No. 5,336,616 ("Livesey"). Applicants respectfully traverse this rejection for the following reasons.

According to the Office Action, Rieck describes a method of extracting fibroblastic growth factor 2 (FGF2) from an ECM by growing endothelial cells, dissolving the cell layer with Triton X-100, exposing the matrix, and extracting the FGF2 with either 2M NaCl or trypsin thus forming a morphogen composition (Office Action at pages 5 to 6). The Office admits that Rieck does not leave the cells intact upon removal, but cites Livesey for disclosing decellularization using salts, detergents (Triton X-100), or enzymes such as trypsin. The Office notes that Livesey "also teaches that with care, cellular removal with enzymes may occur without significant damage to the extracellular matrix (column 9 lines 41-67, emphasis added)" (Action at page 6).

Thus, the Office reasons that it would have been obvious to one of skill in this field to have modified the method of Rieck by using trypsin instead of a detergent, because the Office interprets Livesey as suggesting that this method leaves the matrix intact. That may well be true, but applicants' claimed invention requires that the cells, not the matrix, are kept intact, and it is clear that both Rieck and Livesey are more concerned with keeping the matrix, rather than the cells, intact.

In spite of this clear distinction between applicants' claimed invention and the cited prior art, applicants have amended claim 1 (and 6 for that matter) to clarify that the living cells are removed from the surface using a **non-proteolytic** process. Thus, applicants submit that even when combined, Rieck and Livesey provide no support for an obviousness rejection, and respectfully request that the Examiner reconsider and withdraw this rejection.

Next, the Office has rejected claims 6 to 9 as being unpatentable over Rieck and Livesey in view of Koyama (Nature Biotechnology 1997)("Koyama") and Simpson et al., U.S. Patent Publication No. US 2002/0090725 A1)("Simpson"). Applicants traverse this rejection for the following reasons.

The Office combines Rieck and Livesey as discussed above, but concedes that "Rieck does not teach wherein stimulating the ECM comprises applying an electric potential to the ECM" (Action at page 7). The Office looks to Koyama and Simpson to fill this gap. First, the Office asserts that Koyama discloses that electrical stimulation promotes nerve growth factor secretion from astroglial cells. Second, the Office states that Simpson describes that an electrical field can stimulate movement or conformational changes in a matrix due to the movement of magnetically or electrically sensitive particles, and that this movement can affect the release of compounds or substances such as growth factors from an electroprocessed matrix (citing page 27, paragraph 223)(see Action at pages 7-8).

The Office then concludes that (at page 8):

> one of ordinary skill in the art would have been motivated to use an electric potential to stimulate the secretion of growth factors in the ECM in the method of Rieck because Koyama teaches that electrical stimulation promotes growth factor secretion from cultured cells (which include an ECM) and also because Rieck shows that there is more than one way to extract growth factor from an extracellular matrix (page 37 column 2, line 6). Additional motivation would have been provided by Simpson because Simpson shows that electrical stimulation of the matrix also affects release of compounds from the matrix (page 26 para 222 and page 28 para 230).
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> Therefore, the combined teachings of Rieck, Livesey, Koyama and Simpson render obvious Applicant's invention as claimed.

Applicants strongly disagree with this oversimplified characterization of the prior art and the resulting flawed conclusion of obviousness.

Rieck and Livesey have been discussed above, and Koyama and Simpson are so different from the claimed invention, that they cannot be combined with the methods of Rieck and Livesey as the Office alleges, and even if they were, the result would be something quite different from the claimed invention.

Koyama describes a controlled culture system, in which astroglial cells are directly attached to and grown on a potential-controlled electrode. A small potential is applied to the cells to induce nerve growth factor (NGF) production. See page 164, left column, paragraph 3. Applicants note that claim 6, like claim 1, requires removing living cells intact from an ECM using a non-proteolytic process and leaving the now substantially living cell-free ECM on a surface. After the cell removal step, an electric potential is applied to the ECM to release morphogens. As discussed above, Rieck and Livesey describe methods that involve disrupting cells or treating them with a proteolytic enzyme to remove the cells from ECM. Neither discloses or even suggests removing living cells intact from an ECM using a non-proteolytic process, as required in claim 6. Koyama does not cure this defect. Accordingly, these three references, even when combined, do not provide any motivation to remove **living** cells **intact** from an ECM using a **non-proteolytic process**, as required in the steps of claim 6, and therefore do not render claim 6 obvious.

Further, Koyama's focus is using electric potential to induce the astroglial cells growing on the electrode to produce and secret NGF. Given this focus, one skilled in the art, at best, would have been motivated to apply a potential to living cells, which could produce and secret NGF. He or she would have had no motivation to apply a potential to an ECM from which cells had been removed, because no cells were left to be induced to produce NGF.

To remedy this failing of Rieck, Livesey, and Koyama, the Office cites Simpson and alleges (at page 9):

> Simpson's teaching gives evidence of the release of compounds from the ECM upon treatment with an electric field and Koyama's teaching gives evidence that these compounds (which are secreted by the cells along with the ECM proteins and stimulated to release by an electric field) are growth factors. Therefore, one of ordinary skill in the art would know based on Simpson's teaching of the effect of electrical stimulation on the ECM that the growth factor secreted in the electrical stimulation process of Koyama was also provided by the stimulated ECM as well as the stimulated cells.

Applicants respectfully submit that they have not been able to make much sense of this paragraph. Simpson and Koyama have nothing to do with each other, and Simpson's use of an electroprocessed collagen into which various substances can be introduced for later release provides no incentive to release morphogens from ECM as presently claimed using electrical

stimulation. More importantly, Simpson fails to provide any discussion of the step of "removing living cells from the surface using a non-proteolytic process and leaving the now substantially living cell-free ECM on the surface, wherein the cells remain intact upon removal," which is also lacking in the remaining cited prior art. Thus, adding Simpson to the rejection does not remedy the deficiency in the present obviousness rejection.

It is true that Simpson's collagen can be derived from natural sources, but that is not the point. When used to deliver substances, Simpson's electroprocessed collagen releases substances by diffusion, by degrading over time, or by exposure to light (see page 26, paragraphs 222 and 223). There is no suggestion in these paragraphs to stimulate the release of any substances (e.g., growth factors) using electricity.

The only discussion that applicants have found in Simpson relating to the use of a "magnetic or electric field" to control the release profile of a material in the electroprocessed collagen is when that material is "magnetically sensitive or electrically sensitive" such as magnetically or electrically sensitive particles (see page 28, paragraph 0230, cited by the Office). This mechanism can be used to deliver drugs from the collagen if the drug is somehow mixed into the collagen along with the magnetically or electrically sensitive particles or encapsulated within magnetically or electrically sensitive vesicles within the collagen. These methods use a mechanical change in the collagen matrix to release drugs that have been artificially introduced into the matrix. This is a totally different concept from anything described in any of the other cited references. There are no such particles included in the ECMs described in Rieck or Livesey, and certainly not in the cells described in Koyama, and thus one of skill in this art would never have looked to Simpson to alter the basic methods of Rieck and Livesey. Plainly stated, it makes no common sense.

This is also a totally different concept than claimed in the present invention, in which cells naturally secrete morphogens into an ECM, and the ECM is later electrically stimulated to release those morphogens. As a result, none of the cited references, either alone or in any combination, disclose or even suggest the recited method steps of claim 6 as presently amended. However, as noted above, the main point is that neither Simpson nor Koyama remedies the defect in Rieck and Livesey with respect to the use of a non-proteolytic process to remove living cells intact from the ECM.

In view of the above remarks, applicants submit that claim 6 is patentable over the combination of Rieck and Livesey in view of Koyama and Simpson. Claims 7-9 depend from claim 6 and are thus patentable for at least for the same reasons.

CONCLUSION

Applicants submit that grounds for the rejections asserted by the Examiner have been overcome, and that the pending claims define patentable subject matter. As a result, applicants submit that allowance of this application is proper, and request an early favorable action. Applicants also invite the Examiner to contact them for a telephone interview if the Examiner should still have any questions about the patentability of the pending claims.

No fees are believed due. However, please apply any other required fees to Deposit Account No. 06-1050, referencing attorney docket number 08688-057001.

Respectfully submitted,

Date: Aug. 31, 2007

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